



Attorney Docket No.: 4814.214-US

AF/1652
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A
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Charlotte Johansen Confirmation No: 5761

Serial No.: 09/815,848

Group Art Unit: 1652

Filed: March 23, 2001

Examiner: R. Prouty

For: Antimicrobial Peroxidase Compositions

CERTIFICATE OF MAILING UNDER 37 CFR 1.8(a)

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
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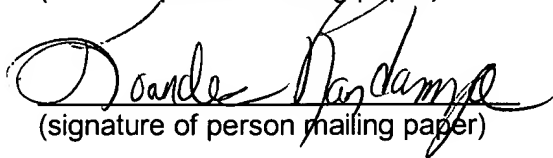
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1. Transmittal of Appeal Brief (in duplicate)
2. Brief on Appeal and a copy of pending claims (in triplicate)

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APPEAL BRIEF

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Alexandria, VA 22313-1450

Sir:

Applicants hereby appeal from the final rejection of claims 31-37, 41-42, 46, 48-49, and 51.

I. REAL PARTY IN INTEREST

The name of the real party in interest in this appeal is Novozymes A/S.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences relating to the instant application.

III. STATUS OF THE CLAIMS

Claims 31-52 remain pending in the application. Claims 1-30 have been canceled. Claims 38-40, 43-45, 47, 50 and 52 have been withdrawn from consideration as being drawn to a nonelected species. A copy of the pending claims is attached in Appendix 1. Claims 31-37, 41-42, 46, 48-49, and 51 are involved in this appeal.

IV. STATUS OF AMENDMENTS

The amendment filed under 37 C.F.R. § 1.116 on June 14, 2004 was considered, but has been stated as not overcoming the final rejection.

V. SUMMARY OF THE INVENTION

The invention relates to methods of killing or inhibiting a microorganism, comprising contacting said microorganism with a composition comprising a peroxidase produced by or derived from *Coprinus* and a hydrogen peroxide or a source of hydrogen peroxide.

VI. ISSUES

The outstanding issues are whether the claims are

- (1) Whether the specification contains an adequate written description for the inventions of claims 31-37, 41, 42, 46, 48, 49, and 51.
- (2) Whether claims 31-37, 41, 42, 46, 48, 49, and 51 are obvious under 35 U.S.C. § 103 over the disclosures of Johansen (WO 96/06532) in view of Schneider et al. (WO 96/10079).

VII. GROUPING OF CLAIMS

For purposes of determining patentability in this appeal, the following claims are grouped together:

Group I: Claim 31, 35-37, 41-42, 46, 48-49, and 51;

Group II: Claim 32; and

Group III: Claim 33-34.

VIII. ARGUMENTS

A. The Specification Contains An Adequate Written Description Of The Invention Of Claims 31-37, 41, 42, 46, 48, 49, and 51

Claims 31-37, 41, 42, 46, 48, 49, and 51 are rejected under 35 U.S.C. 112 "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Specifically, the Office Action stated the following:

These claims are directed to a genus of methods of killing or inhibiting microorganisms using an antimicrobial composition comprising an enhancing agent and a peroxidase. The specification teaches the structural features defining several sub-genuses of such enhancing agents (i.e., those defined in Claims 8 and 10) and the structures of several specific species within these sub-genuses of such enhancing agents. However, the specification fails to describe any other representative species outside of these sub-genuses by any identifying characteristics or properties other than the functionality of being an 'enhancing agent'. Furthermore, the specification teaches only a single representative peroxidase useful in the methods as claimed and the specification fails to describe

any other representative species of peroxidase by any identifying characteristics or properties other than the functionality of being a peroxidase.

This rejection is respectfully traversed.

It is well settled that the test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the claimed subject matter. *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983).

As set forth in Federal Circuit decisions, a specification complies with the written description requirement if it provides "a precise definition, such as by structure, formula, chemical name, or physical properties of the claimed subject matter sufficient to distinguish it from other materials." See, e.g., *University of California v. Eli Lilly and Co.*, 43 U.S.P.Q.2d 1398, 1404 (Fed. Cir. 1997); *Enzo Biochem v. Gen-Probe Inc.*, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002).

Applicants submit that the specification complies with the written description requirement.

The claimed invention is drawn to methods of killing or inhibiting a microorganism, comprising contacting said microorganism with a composition comprising a peroxidase produced by or derived from *Coprinus* and a hydrogen peroxide or a source of hydrogen peroxide. The specification describes a DNA sequence encoding a *Coprinus* peroxidase and one of ordinary skill in the art would appreciate that other *Coprinus* peroxidases would have homologous amino acid sequences. The specification also describes a number of peroxidase enhancing agents. Applicants therefore submit that the specification of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the claimed subject matter.

The Office argues that "multiple different species of an enzyme with unrelated or only low structural similarity to each other within an organism is frequent." This is respectfully traversed.

The Office has provided no evidence that *Coprinus* peroxidases are not structurally similar. To the best of our knowledge, there are only two *Coprinus* peroxidases, one from *Coprinus cinereus* and the other from *Coprinus macrorhizus*. As shown in the attached abstract of Kjalke et al. (Biochim. Biophys Acta, 1992, 1120(3): 248-56), the two peroxidases likely have the same amino acid sequence.

Moreover, claim 32 is drawn to the use of a *Coprinus cinereus* peroxidase, claim 33 is drawn to the use of a peroxidase of a specific *Coprinus cinereus* strain, and claim 34 is drawn to a peroxidase encoded by a specific DNA sequence. The specification also contains an adequate written description of the inventions of these claims.

For the foregoing reasons, Applicants submit that the rejection under 35 U.S.C. 112 is improper. Accordingly, Applicants request that the rejection be reversed.

B. Claims 31-37, 41, 42, 46, 48, 49, and 51 Are Not Rendered Obvious By The Cited References

Claims 31-37, 41, 42, 46, 48, 49, and 51 are rejected under 35 U.S.C. 103 as being unpatentable over Johansen (WO 96/06532) in view of Schneider et al. (WO 96/10079). This rejection is respectfully traversed.

Schneider et al. disclose peroxidase systems comprising an enhancer.

However, Schneider et al. do not teach or suggest the use of peroxidases to kill or inhibit microorganisms.

Johansen discloses compositions capable of killing microbial cells or inhibiting microorganisms. The component that kills or inhibits the microorganisms is a basic protein or peptide of biological origin, e.g., protamine or protamine sulphate. Johansen further discloses that the composition may further comprise a cell wall degrading enzyme and/or an oxidoreductase such as a peroxidase.

In Example 2, Johansen compares the effect of protamine and several enzymes, including a peroxidase enzyme system (i.e., lactoperoxidase and glucose oxidase). The results obtained by Johansen are summarized at page 21 as follows:

It is demonstrated that protamine and protamine sulfate are very effective substances for inhibiting all the tested strains, whereas polyarginine is effective for inhibiting all strains but *Pseudomonas* spp. Apart from the effect of lysozyme on *Listeria monocytogenes*, none of the tested enzyme showed any effect.

The results are shown in Table 4, which show that the peroxidase enzyme system was not effective in killing or inhibiting microorganisms. See column C. A footnote to Table 4 states that "The lactoperoxidase system was effective for maximum 70 hours. The definition of MIC require[s] an inhibition of at least 100 hours." Based on the description of the results, Applicants submit that this phrase should be interpreted to mean that the haloperoxidase system was "active" for 70 hours. It does not mean that the peroxidase system was effective for killing or inhibiting microorganism. Otherwise, Johansen would not have stated that "none of the tested enzyme showed any effect." Thus, Johansen does not teach or suggest the use of peroxidase systems for killing or inhibiting microorganisms.

Moreover, the instant specification shows that *Coprinus* peroxidases have a significant effect in killing or inhibiting microorganisms. See, e.g., the results on page 46 in the specification.

There is no suggestion in the cited references that *Coprinus* peroxidases are superior antimicrobial agents. Thus, these results are surprising and unexpected.

The Office states at page 6 in the Office Action mailed January 14, 2004 that "Johansen clearly suggest that the combination of protamine and a peroxidase system is synergistically effective (see Example 4) in killing or inhibiting microorganisms. Applicants' claims in no way exclude the inclusion of protamine in the compositions. As such the skilled artisan having the disclosures of both Johansen and Schneider would have been motivated to make detergent compositions including enhancing agents, a peroxidase system, protamine and other standard detergent components." This is respectfully traversed.

Johansen provides no data demonstrating that there is synergism between the peroxidase system and protamine. In any event, even if there is synergism, that does not make the use of *Coprinus* peroxidases for killing or inhibiting microorganisms obvious. Johansen states that the peroxidase system alone has no effect. Since a *Coprinus* peroxidase has a significant anti-microbial effect, one of ordinary skill in the art would expect that the combination of a *Coprinus* peroxidase and protamine would have a significantly greater effect than the combination of Johansen's peroxidase system and protamine.


For the foregoing reasons, Applicants submit that the rejection under 35 U.S.C. 103 is improper. Accordingly, Applicants request that the rejection be reversed.

IX. CONCLUSION

For the foregoing reasons, Applicants submit that the rejections are improper. Accordingly, the final rejection of the claims should be reversed.

Respectfully submitted,

Date: November 12, 2004


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APPENDIX

Copy of Claims Involved in the Appeal

31. A method of killing or inhibiting a microorganism, comprising contacting said microorganism with a composition comprising a peroxidase produced by or derived from *Coprinus* and a hydrogen peroxide or a source of hydrogen peroxide.

32. The method of claim 31, wherein the peroxidase is produced by or derived from *Coprinus cinereus*.

33. The method of claim 31, wherein the peroxidase is produced by or derived from *Coprinus cinereus*, IFO 8371.

34. The method of claim 31, wherein the peroxidase is encoded by SEQ ID NO: 1.

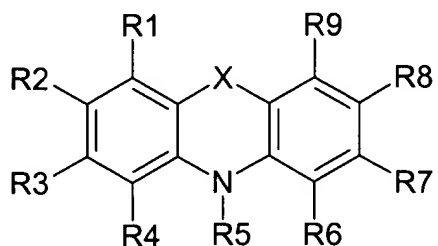
35. The method of claim 31, wherein the source of hydrogen peroxide is an enzymatic hydrogen peroxide-generating system.

36. The method of claim 35, wherein the enzymatic hydrogen peroxide-generating system is glucose oxidase/glucose, hexose oxidase/hexose, L- or D-amino acid oxidase/L- or D-amino acid, or lactate oxidase/lactate.

37. The method of claim 31, wherein the composition further comprises an electron donor.

38. The method of claim 31, wherein the composition further comprises a water-soluble halide or thiocyanate salt.

39. The method of claim 31, wherein the composition further comprises a compound of the following formula:

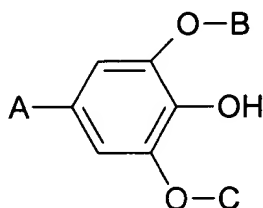


wherein X is (-O-) or (-S-), and R^1-R^9 , which may be identical or different, independently are any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy, and esters and salts hereof, carbamoyl, sulfo, and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C_1-C_{14} -alkyl, C_1-C_5 -alkoxy, carbonyl- C_1-C_5 -alkyl, aryl- C_1-C_5 -alkyl; which carbamoyl, sulfamoyl, wherein the amino groups may be unsubstituted or substituted once or twice with R^{10} ; and which phenyl may be unsubstituted or substituted with one or more R^{10} ; and which C_1-C_{14} -alkyl, C_1-C_5 -alkoxy, carbonyl- C_1-C_5 -alkyl, and aryl- C_1-C_5 -alkyl groups may be saturated or unsaturated, branched or unbranched, and may be unsubstituted or substituted with one or more R^{10} ; wherein R^{10} is any of the following radicals: halogen, hydroxy, formyl, carboxy and esters or salts thereof, carbamoyl, sulfo and esters or salts thereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidin-1-yl, C_1-C_5 -alkyl, C_1-C_5 -alkoxy; which carbamoyl, sulfamoyl, and amino groups may be unsubstituted or substituted once or twice with hydroxy, C_1-C_5 -alkyl, or C_1-C_5 -alkoxy; and which phenyl may be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C_1-C_5 -alkyl, and C_1-C_5 -alkoxy groups may be saturated or unsaturated, branched or unbranched, and may be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts thereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; or wherein two of R^1-R^9 form a group -B-, in which B is any of the following the groups: $(-CHR^{10}-N=N-)$, $(-CH=CH-)_n$, $(-CH=N-)_n$ or $(-N=CR^{10}-NR^{11}-)$, in which groups n is an integer of from 1 to 3, R^{10} is a substituent group as defined above and R^{11} is defined as R^{10} .

40. The method of claim 31, wherein the composition further comprises a compound selected from the group consisting of 10-methylphenothiazine, phenothiazine-10-propionic acid, N-hydroxysuccinimide phenothiazine-10-propionate, 10-ethyl-phenothiazine-4-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl phenothiazine-10-propionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methylpiperazin-1-yl)propyl)phenothiazine, 10-(2-pyrrolidin-1-yl-ethyl)phenothiazine, 2-methoxy-10-methyl-phenothiazine, 1-methoxy-10-methylphenothiazine, 3-methoxy-10-methylphenothiazine, 3,10-dimethylphenothiazine, 3,7,10-trimethylphenothiazine, 10-(2-hydroxyethyl)phenothiazine, 10-(3-hydroxypropyl)phenothiazine, 3-(2-hydroxyethyl)-10-methylphenothiazine, 3-hydroxymethyl-10-methylphenothiazine, 3,7-dibromophenothiazine-10-propionic acid, phenothiazine-10-propionamide, chlorpromazine, 2-chloro-10-methylphenothiazine, 2-acetyl-10-methylphenothiazine, 10-methylphenoxazine, 10-

ethylphenoxazine, phenoxazine-10-propionic acid, 10-(2-hydroxyethyl)phenoxazine and 4-carboxyphenoxazine-10-propionic acid.

41. The method of claim 31, wherein the composition further comprises a compound of the following formula:



wherein A is -D, -CH=CH-D, -CH=CH-CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D; wherein D is -CO-E, -SO₂-E, -N-XY, or -N⁺-XYZ; E is -H, -OH, -R, or -OR, and X, Y and Z may be identical or different and are -H or -R; wherein R is C₁-C₁₆ alkyl, optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and are C_mH_{2m+1}; wherein 1 ≤ m ≤ 5.

42. The method of claim 31, wherein the composition further comprises acetosyringone, methylsyringate, ethylsyringate, propylsyringate, butylsyringate, hexylsyringate, or octylsyringate.

43. The method of claim 31, wherein the composition further comprises ammonium halide, calcium halide, lithium halide, potassium halide, or sodium halide.

44. The method of claim 31, wherein the composition further comprises ammonium iodide, calcium iodide, lithium iodide, potassium iodide, or sodium iodide.

45. The method of claim 31, wherein the composition further comprises ammonium thiocyanate, potassium thiocyanate, or sodium thiocyanate.

46. The method of claim 31, wherein the microorganism is present in laundry.

47. The method of claim 31, wherein the microorganism is present on skin, hair, mucous membranes, teeth, wounds, bruises or in the eye or oral cavity, of a human or animal.

48. The method of claim 31, wherein the composition is in the form of a soaking, washing or rinsing liquor.

49. The method of claim 31, wherein the composition is a liquid composition.
50. The method of claim 31, wherein the composition is a mouth wash, an antiinflammatory liquid, a perspirant, a deodorant, or a nasal spray.
51. The method of claim 31, wherein the composition is a solid composition.
52. The method of claim 31, wherein the composition is an eye ointment, an anti-inflammatory ointment, a foot bath salt, a perspirant, or a deodorant.



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Comparison of structure and activities of peroxidases from *Coprinus cinereus*, *Coprinus macrorhizus* and *Arthromyces ramosus*.

Kjalke M, Andersen MB, Schneider P, Christensen B, Schulein M, Welinder KG.

Institute of Biochemical Genetics, University of Copenhagen, Denmark.

Initial structural and kinetic data suggested that peroxidases from *Coprinus cinereus*, *Coprinus macrorhizus* and *Arthromyces ramosus* were similar. Therefore they were characterized more fully. The three peroxidases were purified to RZ 2.5 and showed immunochemical identity as well as an identical M(r) of 38,000, pI about 3.5 and similar amino acid compositions. The N-termini were blocked for amino acid sequencing. The peroxidases had similar retention volumes by anion-exchange and gel-filtration chromatography. All peroxidases showed multiple peaks by Concanavalin A-Sepharose chromatography. The Concanavalin A-Sepharose profiles were different and depended furthermore on a fermentation batch. Tryptic peptide maps were very similar except for one peptide. This peptide contained an N-linked glycan composed of varying ratios of glucosamine and mannose for the three peroxidases. Rate constants and their pH dependence were the same for the three peroxidases using guaiacol or iodide as reducing substrates. We conclude that peroxidases from *Coprinus cinereus*, *Coprinus macrorhizus* and *Arthromyces ramosus* are most likely identical in their amino acid sequences, but deviate in glycosylation which, apparently, has no influence on the reaction rates of the enzyme. We suggest, that the *Coprinus* fungi express one peroxidase only in contrast to the lignin-degrading white-rot Basidiomycetes, which produce multiple peroxidase isozymes.

PMID: 1576150 [PubMed - indexed for MEDLINE]

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